***DOTTORATO DI RICERCA IN BIOLOGIA CELLULARE E DELLO SVILUPPO***

**41° Cycle**

**Project proposal for a PhD scholarship**

**Main research line**

**Title of the research: Anti-cancer properties of new Naphatalimide-derivates in glioblastoma cancer stem cells: possible dual role as M2 muscarinic receptor agonist and as a de-methylase inhibitor**

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**Summary**

Glioblastoma (GB) is the most malignant human brain tumor characterized by heterogeneous cell populations, including undifferentiated cells defined GB Stem cells (GSCs), responsible for the beginning of the neoplastic process and recurrence formation. Previous studies demonstrated that the activation of M2 muscarinic receptor (M2 mAChR) caused a significant decrease of cell proliferation and survival in both GSCs and GB cell lines. Recently, we have evaluated a new synthetic M2 dualsteric agonist, Iper-8-naphthalimide (N-8-Iper), a new dualsteric agonist derived by naphatalimide. It affects GSCs survival, inducing cytotoxic effects and DNA damage in GSCs (in particular in p53 mutated cell line). We have demonstrated that N-8-Iper, can counteract drug resistance in two GSC lines, GB7 cells and the more resistant G166 cells. Recently it has been demonstrated that naphthalimides possess potent antitumor activity against different tumors. One of the important features of naphthalimides is their ability to target biological macromolecules, especially nucleic acids. The planar nature of the aromatic core suggests that the molecule should intercalate itself between base pairs of DNA to induce DNA double-strand breaks (DSBS), a behavior that has been assumed in many cases. DNA methylation has also been reported to promote DNA DSBS, DNA or RNA modifications playing a critical roles in regulating gene expression. The most prevalent mRNA modification, N6-methyladenosine (m6A), governs the fates of modified mRNA molecules and affects many important biological processes including development and stem cell differentiation. Moreover, DNA/RNA demethylase can act as an oncogenic factor to trigger the progression of several human cancers, such as leukemia, glioblastoma, and neuroblastoma. Therefore it has been proposed that inhibitors of demethylase enzymes may play a strategic role in controlling the tumor progression and regulating tumor cell proliferation and differentiation. N8-iper was studied in glioblastoma as a selective ligand for M2 muscarinic receptors, but its de-methylase inhibitor function has not yet been evaluated. This project will compare the effects of N8-ipernaphatalimide and its modified fluorescent form, named Fluo N8-ipernapthalimide as antitumor drugs. In particular we would like to dissect which effects (cytotoxic and genotoxic) are dependent on M2 agonist function or de-methylase inhibitor.

**Pertinent Publications of the proponent (last 5 years)**

1. Ilaria Cristofaro, Chiara Limongi, Paola Piscopo, Alessio Crestini, Claudia Guerriero, Mario Fiore, Luciano Conti, Annamaria Confaloni, Ada Maria Tata. M2 Receptor Activation

Counteracts the Glioblastoma Cancer Stem Cell Response to Hypoxia Condition. Int. J Mol Sci., 2020, 21(5). pii: E1700. doi: 10.3390/ijms21051700.

2. Ilaria Cristofaro, Francesco Alessandrini, Zaira Spinello, Claudia Guerriero, Mario Fiore, Luciana Dini, Luciano Conti and Ada Maria Tata . Cross interaction between M2 muscarinic receptor and Notch1/EGFR pathway in human glioblastoma cancer stem cells: effects on cell cycle progression and survival. Cells, 2020, 9, 657; doi:10.3390/cells9030657

3. Alejandro J. Español, Agustina Salem,, María Di Bari, Ilaria Cristofaro, Yamila Sanchez, Ada Maria Tata, María E. Sales. The metronomic combination of paclitaxel with cholinergic agonists inhibits triple negative breast tumor progression. Participation of M2 receptor subtype. PlosOne 2020, 15(9): e0226450. https://doi.org/10.1371/journal.pone.0226450.

4. Anna Maria Lucianò and Ada Maria Tata. Functional characterization of cholinergic receptors in melanoma cells. Cancers, 2020, 12; 3141. doi.org/10.3390/cancers12113141

5. Anna Maria Lucianò, Elisa Perciballi, Mario Fiore, Donatella Del Bufalo, Ada Maria Tata. The combination of the M2 muscarinic receptor agonist and chemotherapy affects drug resistance in neuroblastoma cells" Int J Mol Sci. 2020, 21, 8433, doi.org/10.3390/ijms21228433.

6. Maria Di Bari, Vanessa Tombolillo, Francesco Alessandrini, Claudia Guerriero, Mario Fiore, Italia A. Asteriti, Emilia Castigli, Miriam Sciaccaluga, Giulia Guarguaglini, Francesca Degrassi, Ada Maria Tata 'M2 muscarinic receptor activation impairs mitotic progression and bipolar mitotic spindle formation in human glioblastoma cell lines' Cells 2021, 10(7):1727. doi: 10.3390/cells10071727.

7. Claudia Guerriero, Carlo Matera, Donatella del Bufalo, Marco De Amici, Luciano Conti, Clelia Dallanoce and Ada Maria Tata. Combined treatments with dualsteric agonist N-8-Iper plus chemotherapy drugs affect drug resistance in glioblastoma stem cells. Cells 2021, 10(8):1877. doi: 10.3390/cells10081877.

8. Taggi Marilena; Kovacevic Andjela; Capponi Chiara; Falcinelli Marta; Cacciamani Veronica; Vicini Elena; Canipari Rita; Tata Ada Maria. The activation of M2 muscarinic receptor inhibits cell growth and survival in human epithelial ovarian carcinoma. J Cell Biochem, 2022, doi: 10.1002/jcb.30303.

9. Piovesana R. Reid AJ, Tata A.M. Alternative functions of Neurotransmitters in glial cells: emerging role of Cholinergic Receptors in Schwann Cell Development and Plasticity. Biomedicines, 2023 11(1):41. doi: 10.3390/biomedicines11010041.

10. Guerriero, C.; Manfredelli, M.; Matera, C.; Iuzzolino, A.; Conti, L.; Dallanoce, C.; De Amici, M.; Trisciuoglio, D.; Tata, A.M. M2 Muscarinic Receptor Stimulation Induces Autophagy in Human Glioblastoma Cancer Stem Cells via mTOR Complex-1 Inhibition. Cancers 2023,. 20;16(1):25. https://doi.org/ 10.3390/cancers16010025

11. Claudia Guerriero, Rachele Fanfarillo, Patrizia Mancini, Valentina Sterbini, Giulia Guarguaglini, Luigi Sforna, Antonio Michelucci, Luigi Catacuzzeno, Ada Maria Tata. M2 muscarinic receptors negatively modulate cell migration in human glioblastoma cells. Neurochem Int, 174:105673, 2024, https://doi.org/10.1016/j.neuint.2023.105673.